Summary

Overweight and obesity have adverse metabolic effects on blood pressure, blood lipids and insulin resistance, consequently increasing the risk of chronic diseases such as type 2 diabetes, cardiovascular diseases, and even certain forms of cancer. Together with abdominal obesity, insulin resistance plays a principal role in initiating and perpetuating the pathological manifestations of the metabolic syndrome. Obesity results from the combined effects of genes, environment and lifestyle. In this context, an understanding of these effects of lifestyle and genes on obesity and also their interactions is important to provide a basis for determining the role they could have on the development and prevention of obesity. The main aim of the current thesis was to investigate the association of several candidate genes (the beta-2 adrenergic receptor gene [*ADRB2*], the nitric oxide synthase-3 gene [*NOS3*] and the apolipoprotein-B gene [*APOB*]) with adiposity, and to assess whether the previously identified common variants in the fat mass and obesity-associated *(FTO)* gene, near the insulin induced gene 2 *(INSIG2)* and the melanocortin 4 receptor *(MC4R)* gene identified in GWA studies were associated with adiposity and insulin resistance in European-American (EA) and African-American (AA) youth in both cross-sectional and longitudinal studies. We used data from the Georgia Cardiovascular Twin (Georgia CV Twin), the Lifestyle, Adiposity and Cardiovascular Health in Youths (LACHY) and the Adiposity Prevention through Exercise (APEX) studies in the cross-sectional investigations, and the Georgia CV Twin and Blood Pressure Stress cohorts in the longitudinal studies. Furthermore, the potential interaction of these common variants with ethnicity, gender or lifestyle behaviors (diet and physical activity) was investigated. In addition, we estimated the influence of genetic and environmental factors on fasting and 2-h glucose and insulin in a multivariate fashion in a large sample of UK female twins.

In **Chapter 2**, we aimed to investigate the influence of and interaction between lifestyle behaviors (diet and physical activity [PA]) and single nucleotide polymorphisms (SNPs) in obesity candidate genes (*ADRB2, APOB* and *NOS3*) on general and central adiposity in the LACHY study. Six hundred and twenty-one EA and AA youth aged 13-19 years were classified by ethnicity (49% AA), gender (45% male) and socio-economic status (SES). Along with body mass index (BMI) and waist circumference, which cannot distinguish between fat and lean mass, more accurate indices for general and central adiposity were used, such as percent body fat (%BF)

based on dual-energy X-ray absorptiometry (DXA) as well as visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAAT) measured by magnetic resonance imaging (MRI). PA and dietary intake with up to 7 24-hour recalls were reported for all subjects. We found that reported energy intake and vigorous PA (VPA) were negative predictors of %BF and SAAT. Carriers of the *NOS3* Asp298 allele had higher %BF only in the presence of an adverse environment (Low SES). Compared to the most common *NOS3* haplotype, homozygotes for haplotype A-non4r-Asp had 6.1% higher %BF. Significant interactions were revealed between the *ADRB2* ARg16Gly SNP and VPA on VAT such that Gly16 homozygotes may benefit less from increased VPA to reduce their weight. Thus, genetic susceptibility to increased general and central adiposity is dependent on several factors, such as SES and vigorous exercise. Improved understanding of such joint effect of genes and lifestyle on adiposity may offer new insights into the etiology of obesity and provide new avenues for personalized prevention and treatment.

In **chapter 3**, we assessed the influence of *FTO* variant rs9939609 on adiposity, insulin resistance, energy intake and physical activity in EA and AA youth in a cross-sectional study. One thousand, nine hundred and seventy-eight youth (48.2% EAs, 47.1% male, mean age 16.5 years) had measures of anthropometry. %BF was measured by DXA, VAT and SAAT by MRI. Energy intake and physical activity were measured through self report from up to 7 24-hour recalls. Physical activity was also measured by accelerometry. We found that *FTO* rs9939609 was significantly associated with BMI (P=0.01), weight (P=0.03) and waist circumference (P=0.04), with per-allele effects of 0.4 kg/m^2, 1.3 kg and 0.8 cm, respectively. No significant association was found between rs9939609 and %BF, VAT, SAAT or insulin resistance (P>0.05), or between rs9939609 and energy intake or vigorous physical activity (P>0.05). No significant interactions of rs9939609 with ethnicity, gender, energy intake or physical activity were observed (P>0.05). In conclusion, the *FTO* variant rs9939609 is modestly associated with BMI and waist circumference, but not with energy intake or physical activity. Moreover, these effects were similar for EAs and AAs.

Genome-wide association (GWA) studies found that common variants near the *MC4R* gene were associated with obesity. In **chapter 4,** the influence of these SNPs (rs17782313 and rs17700633) on general and central adiposity was assessed in EA and AA youth. In 1890 youth (49.1% EA, 45.6% male, mean age 16.7 years), we examined the associations of the rs17782313 and rs17700633 with anthropometry,

%BF, VAT and SAAT. Interaction of the SNPs with ethnicity and gender was investigated and haplotype analyses conducted. Rs17782313 was significantly associated with weight (P=0.02) and waist circumference (P=0.03) in all subjects, and with BMI (P=0.002) in females. In females, rs17700633 was significantly associated with %BF (P=0.001), VAT (P<0.001) and SAAT (P<0.001). Rs17700633 was significantly associated with fasting insulin and HOMA, but the significance attenuated after adjustment for %BF. These findings were confirmed by haplotype analysis. No significant interactions of the variants with ethnicity were found for any of the adiposity phenotypes. The relatively large effect of these common variants near *MC4R* on general and central adiposity in childhood, especially in girls, could prove helpful in elucidating the molecular mechanisms underlying the development of obesity in early life.

GWA studies identified several common variants for obesity: rs9939609 in *FTO*, rs7566605 near *INSIG2* and both rs17782313 and rs17700633 near the *MC4R* gene. In **chapter 5,** we assessed the influence of these polymorphisms on development of adiposity in EA and AA youth in two ongoing longitudinal studies including 986 and 606 participants, respectively. Individual growth curve modeling was conducted separately in the two studies. We tested the effect of the SNPs on levels and increase with age (i.e.,slope) of weight, BMI, waist circumference and skinfolds from childhood to adulthood, and potential moderation by ethnicity or gender. Beta coefficients computed in the two studies were pooled using meta-analysis. We found that rs9939609 was associated with levels of BMI (P=0.01), weight (P=0.04) and waist circumference (P=0.04). Rs17782313 was associated with triceps skinfolds (P=0.02). Significant interactions of rs17700633 with gender were observed on subscapular-, suprailiac- and sum of skinfolds, with significant associations limited to males (Ps<0.05). No significant interactions with ethnicity were found. Only one effect on the slope was observed, rs17700633 showed a significant interaction with age on triceps (P=0.04). In conclusion, in two longitudinal studies of EA and AA youth, we replicated the effect of *FTO* and common variants near *MC4R* on general and central adiposity. These variants did not affect the increase with age of adiposity from childhood to adulthood with one exception. Common variants for obesity identified in GWA studies have detectable but modest effects on growth curves for adiposity in EA and AA youth.

There are some major strengths to the abovementioned studies. They involved EA as well as AA youth allowing the investigation of a potential interaction of the

investigated SNPs with ethnicity. To date, most GWA and replication studies on obesity were conducted in Europeans, with only a few including Asians and AAs. The association between common variants in *FTO* and near *MC4R* and BMI has been firmly established in Europeans, but the findings in populations of African ancestry are inconsistent for both *FTO* and *MC4R*. Our studies provided evidence of the association between these common variants and obesity in both EAs and AAs. Another major strength is that, in addition to anthropometric measures, we included more accurate measurements of general (%BF), visceral (VAT) as well as subcutaneous adiposity (SAAT). In addition to assessing the association of the common variants with adiposity in cross-sectional studies, we also performed individual growth curve modeling in two longitudinal studies, which allowed us to obtain better insight into the influence of common genetic variants on the development of adiposity from childhood into adulthood. Longitudinal designs also have superior power to detect genetic effects on the trait levels compared to cross-sectional studies.

Twin and family studies have shown the importance of genetic factors influencing fasting and 2 h glucose and insulin levels. However, the genetics of the physiological response to a glucose load has not been thoroughly investigated. In **chapter 6,** we studied 580 monozygotic and 1937 dizygotic British female twins from the TwinsUK Registry. The effects of genetic and environmental factors on fasting and 2 h glucose and insulin levels were estimated using univariate genetic modelling. Bivariate model fitting was used to investigate the glucose and insulin responses to a glucose load, i.e. an oral glucose tolerance test (OGTT). The genetic effects on fasting and 2 h glucose and insulin levels ranged between 40% and 56% after adjustment for age and BMI. Exposure to a glucose load resulted in the emergence of novel genetic effects on 2 h glucose independently of the fasting level, accounting for about 55% of its heritability. For 2 h insulin, the effect of the same genes that already influenced fasting insulin was amplified by about 30%. In conclusion, exposure to a glucose challenge uncovers new genetic variance for glucose and amplifies the effects of genes that already influence the fasting insulin level. A recent meta-analysis of nine GWA studies found that of the loci associated with 2 h glucose some were (*ADCY5,GCKR, TCFL2)* but some were not (*GIPR and VPS13C)* significantly associated with fasting glucose, which confirmed our findings of both shared and specific genetic factors for fasting and 2-h glucose. Finding the genes acting on 2 h glucose independently of fasting glucose may offer new etiological insight into the risk of cardiovascular events and death from all causes.

In **Chapter 7**, the main findings of this thesis are reviewed and interpreted. Overall, the findings presented in this thesis supported the associations of the common variants identified in GWA studies with common obesity, whereas recent GWA findings confirmed that exposure to a glucose load uncovers new genetic variance. Furthermore, I discussed some methodological issues. For example, the advantages of longitudinal study designs to obtain better insight into the influence of common genetic variants on the development of adiposity over time, and its superior power compared to cross-sectional studies. I reviewed the GWA studies that identified loci robustly associated with general and central obesity so far. The discoveries of GWA studies have provided valuable new insights into the genetic architecture of complex diseases. However, the identified variants associated with BMI or waist circumference so far explain only a very small proportion of the total genetic effect of these traits. This raises the question where the missing heritability might be found. Consequently, current implications for clinical practice and public health of these GWA findings are limited. To conclude, I provided my perspective on future directions that may aid discovery of more susceptibility loci and improve the understanding of physiological and etiological mechanisms whereby these loci confer susceptibility to overweight and obesity.